Sample preparation and SANS measurements of the multi-domain protein Hef with two site-specifically deuterated domains

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More than half of eukaryotic proteins are multi-domain proteins (MDPs) containing intrinsically disordered regions (IDRs). Although IDRs are crucial for biological functions like substrate recognition, their high mobility severely hinders conventional structural analyses via X-ray crystallography, cryo-EM, and NMR. To overcome this, we utilized small-angle scattering, which has virtually no constraints on the sample conditions, to elucidate the structures and dynamics of MDPs and their internal IDRs. We focused on Hef, an MDP consisting of Helicase and Nuclease domains connected by an IDR (Fig. 1(a)), and analyzed its solution structure. The SAXS profile of Hef reflects all three components. While SAXS provides overall conformational data, directly observing the IDR within the full-length molecule would clarify the relationship between its flexibility and the global structure. In SANS, a 75% deuterated protein is invisible (matched out) in 100% D_2O [1,2]. Utilizing this, we previously succeeded in preparing and measuring two Hef variants in which either the Helicase or Nuclease domain was selectively 75% deuterated. These samples were prepared by joining the respective protonated and deuterated fragments via enzymatic protein ligation (Fig. 1(b)). In this study, we attempted to prepare a doubly ligated Hef sample where both domains were 75% deuterated while leaving the IDR protonated (Fig. 1(b)). This preparation is highly challenging due to the need for ligation at two distinct sites. By screening ligation enzymes and selecting appropriate ligation points to optimize efficiency, we successfully prepared the target sample. Because Hef tends to aggregate in a concentration-dependent manner, SANS data were collected using SEC-SANS to prevent aggregation, with the elution concentration adjusted to ~0.5 mg/mL. However, because the visible IDR (~100 residues) accounts for only 1/8 of the full-length Hef (~800 residues), the scattering signal was extremely weak. Despite long exposure times (17.5 hours total at 4 m and 10.5 hours total at 1 m camera length), data suitable for structural analysis could not be obtained (Fig. 2). Future work will optimize aggregate-free solution conditions at higher protein concentrations to achieve high-quality data.

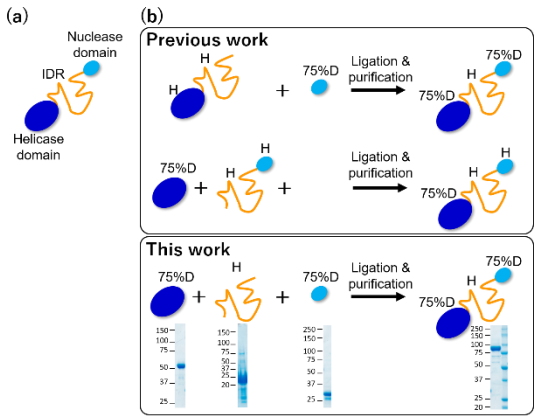

Fig. 1. Schematic view of domain structure (a) of Hef and preparation of domain-selective deuteration by protein ligation (b).

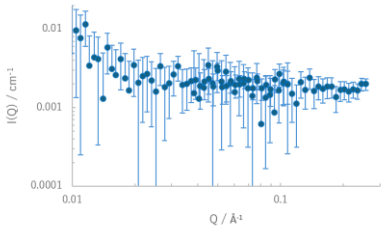

Fig. 2. SANS profile of Hef with both domains deuterated, measured in 100% D_2O buffer. Only scattering from the protonated IDR should be observed.

References

[1] R. Inoue et al., *Sci. Rep.* 11,2555 (2021).
[2] R. Inoue et al., *Biophys. Physicobiol.* 18, 16 (2021).